Abstract No. Stoc0022

Structure of a Cholesterol-Binding Protein Deficient in Niemann-Pick Type C2 Disease

N. Friedland (UMDNJ-RWJMS, HHMI), H.-L. Liou (UMDNJ-RWJMS), P. Lobel (UMDNJ-RWJMS), and A. Stock (UMDNJ-RWJMS, HHMI)

Beamline(s): X4A

Introduction: Niemann-Pick disease type C2 (NP-C2) is a fatal hereditary disease characterized by accumulation of LDL-derived cholesterol in lysosomes. The disease results from a deficiency NPC2, a lysosomal protein that has been shown to bind cholesterol (for review see ref. 1).

Methods and Materials: Initial attempts to express recombinant mammalian NPC2 proteins were unsuccessful most likely due to the presence of 6 Cys residues involved in disulfide bonds. We therefore sought an endogenous source of NPC2 protein. Bovine NPC2 protein (bNPC2) is present in reasonable quantities in milk and this source allowed for purification of quantities sufficient for crystallographic studies. Deglycoslylation of bNPC2 with EndoHf enzyme was necessary for crystallization. The structure of bNPC2 was determined by single-wavelength anomalous dispersion (SAD) methods and was refined to 1.7 angstroms resolution. Platinum tetrachloride was used as a heavy atom derivative.

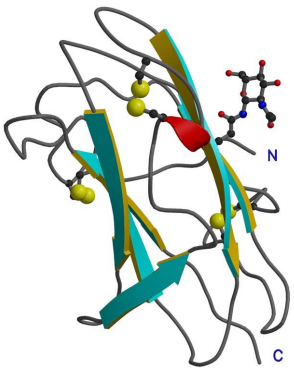
Results: We have investigated the functional and structural properties of the NPC2 protein in order to gain insight into its role in cholesterol transport. We have determined that human NPC2 binds the cholesterol analogue dehydroergosterol (DHE, ergosta-5,7,9(11),22-tetraen-3β-ol) with submicromolar affinity at both neutral and acidic pH. We have determined the high-resolution crystal structure of bovine apoNPC2. The protein has an immunoglobulin-like fold, but with a different pattern of disulfide bridges. The structure reveals a loosely packed region in the protein interior that appears to be an incipient cholesterol-binding site. NPC2 has structural similarity to the dust mite allergen proteins Der p 2 and Der f 2, proteins of unknown physiological function that have been shown by crystallographic analysis to bind hydrophobic ligands of unknown identity within their hydrophobic interiors. The proposed mechanism for cholesterol binding to NPC2 is fundamentally similar to that observed in Der p 2.

Conclusions: The structure of bovine NPC2 together with the previous structure of Der p 2, suggest a new class of sterol binding proteins. Previously characterized sterol binding proteins are typically alpha/beta proteins that have large, preformed sterol binding sites that exist in the absence of ligand. In contrast, NPC2 has an all-beta fold and lacks a substantial cavity. Instead, a loosely packed region of the hydrophobic interior apparently represents an incipient binding pocket that must expand substantially via a conformational change in the protein in order to accommodate a cholesterol ligand.

Acknowledgments: We thank Craig Ogata for assistance with data collection at HHMI beamline X4A at the National Synchrotron Light Source at Brookhaven National Laboratory; Istvan Sohar and Henry Lackland for advice and assistance in protein purification and analytical protein chemistry; Rob Jordan, Richard Leidich, Eva and Brian Fulmer for help in obtaining raw milk; and Judy Storch for helpful discussions regarding initial development of the binding assay. This work was supported in part by grants from the National Institutes of Health (NS37918) and the Ara Parseghian Medical Research Foundation (P.L.). A.M.S. is an associate investigator of the Howard Hughes Medical Institute.

References:

1. S. Naureckiene, D.E. Sleat, H. Lackland, A. Fensom, M.T. Vanier, R. Wattiaux, M. Jadot and P. Lobel 2000 "Identification of HE1 as the Second Gene of Niemann-Pick C Disease," Science ***290***, *2298-2301*.



Ribbon representation of bovine NPC2. Three disulfide bonds are highlighted with yellow sulfur atoms. The N-acetyl glucosamine moiety modifying Asn39 is shown in ball and stick representation.